Report for: Chief of Naval Research
Attn: Dr. Roger D. Reid, Microbiology Branch (Code 443)
Office of Naval Research
Department of the Navy
Washington 25, D. C.

Project: NR 135-233 entitled "The biological effects of high dosage X-radiation upon dividing, non-dividing cells and other stages of Paramecium using the newly developed Nylon radiation chamber method."

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Report

Clones of seven species of Paramecium were irradiated in gradually increased dosages up to 600 kiloroentgen using Nylon syringes as irradiation chambers. Specimens were cultivated in desiccated lettuce medium with Aerobacter aerogenes as the bacterial food source and irradiated generally at the peak or slightly beyond the logarithmic growth phase. The new device* (Fig. 1) consists of a piece of 1" machined Plexiglas with two ice-wells and holes for four Nylon hypodermic syringes of 2 cc capacity and graduated in units of one-tenth of a cc. A tightly fitting machined Lucite cap is applied over the tapering end of each syringe. The syringes and holder, unlike glass, absorb very little irradiation, eliminate air from the irradiation chamber and permit the introduction of various substances to be tested during irradiation. Accurate sampling of specimens after intervals of irradiation without changing the depth of the medium is also a desirable feature. Four syringes, each containing 200 counted cells, are irradiated simultaneously. The device and method appear to be ideal for the study of the biological effects of all

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* The Plexiglas syringe holders and Lucite syringe caps were constructed by Mr. Michael Troisi, Instrument Maker, Temple University.

kinds of irradiation upon Paramecium (Fig. 2) and should prove to be useful for similar studies with other microorganisms. For details in its use see previous report.

Each experiment involved 800 specimens (200 per syringe in 2 cc of fluid). After each x-ray dosage - commonly in steps of 50,000 roentgen - countable numbers of cells (usually 10) were expressed from the syringes, placed in sterile Pyrex spot plates in moist chambers and observations continued periodically to 48 hours.

Variations in x-ray susceptibility exist not only among species but within a species. After each experiment a graph was made in which the percentage of irradiated survivors was computed against x-ray dosage so as to obtain an LD 50 (lethal dosage at which 50% of irradiated organisms are killed) for 24 and 48 hour periods. Under fairly uniform cultural and x-ray conditions, it is observed that the species may be arranged in sensitivity to x-rays with P. calkinsi and P. multimicronucleatum the most resistant (LD 50: approximately 400 Kr.) and the smallest, P. trichium the most sensitive (LD 50: 170 Kr.) for 24 hour periods as follows: P. calkinsi, P. multimicronucleatum, P. bursaria, P. caudatum, P. aurelia, P. polycaryum and P. trichium. Generally, it is to be noted that members of the species of smallest size, which are most susceptible to x-radiation, are at one end of the range and that members of the species of larger size and which are most resistant are at the opposite end of the range.

X-ray susceptibility in paramecia-cells is dependant not only upon the concentration of bacteria and dissolved oxygen in the medium, the chemical constitution of the culture fluid and changes brought upon it as a result of ionizing radiations (such as the production of hydrogen peroxide) but also the phases and stages of growth of individual paramecia.

When the results of many experiments are plotted to yield per cent survival curves, such curves are not frequently typically sigmoid as is claimed to be the case for most irradiated organisms. When large masses of data are compiled and treated statistically, one obtains a curve that is so steep in approaching lethality as to be almost vertical. However, before the steep drop, commonly two peaks occur in the curve. These peaks or variations from the typical curve are greater than the biological variation expected and must indicate sensitivity levels and differences in specimens of a clone being irradiated. These sensitivity thresh-holds, plus conditions of irradiation and the fact that many immobilized specimens may appear seemingly dead for hours through the 24 hour period only to recover later from irradiation effects may explain the difficulty in obtaining consistently uniform LD values.

Instead of the conventional survival curve in which percentage of irradiated survivors is computed against x-ray dosage (which was done for each experiment) still another type of plot was constructed for each experiment. For this type, a series of curves is constructed for each x-ray dosage in an experiment, beginning at 50,000 roentgen and ending in 500,000 r in steps of 50,000 r. On the ordinate is represented percentage of survivors and on the abscissa is represented time through a 48 hour period. Thus one can take into account those irradiated animals which are seemingly dead (immobilized and inactive) but which recover and occasionally reproduce within the 48 hour period. Such a series of 10 curves per experiment for each dosage are much more meaningful in studying and interpreting lethality, depression of fission rate, recovery, reproduction and growth. The comparative curves shown on a single graph are of interest in that they show plateaus - the greater the x-ray dosage, the longer the plateau. Then there may occur a gradual decline showing no recovery but ending in death in the very high dosages or a series of spaced ascending

curves - depending on the dosage - resulting in recovery and in some instances, reproduction in a 48 hour period. The most representative of the graphs before me will be selected for inclusion in the manuscript for publication.

One important part of the study is concerned with the use of various reagents and chemicals which were placed in the syringes with normal paramecia to determine whether or not the chemicals gave preprotection against x-radiation (as compared with controls) or if they sensitized and killed the paramecia. Both aspects of the problem are important as indicated below.

In the project last year, it was discovered that non-toxic hematoporphyrin in concentrations of 1:10,000 to 1:40,000 caused 100% mortality in 12 hours when irradiated with only 100,000 roentgen; all controls survive this dosage and it should be remembered that the LD 50 for 24 hours is approximately 340 Kr. for P. caudatum. To obtain more precise end points, Dr. Figge and I performed a series of experiments this summer on greater dilutions of hematoporphyrin with lesser, more graduated steps of dosages (beginning with 5000 r dosages and increased in identical steps to 100,000 r). We have been able to obtain more precise data so that it is now possible to construct survival curves which will be reproduced in the published paper.

It is of interest to report that the discovery in which x-rays with porphyrin sensitize and kill the cells with ordinarily sublethal x-ray dosage has practical advantages. There is evidence that cancer cells take in porphyrin while other cells do not. Tests are under way with over a dozen patients injected with porphyrin. As with paramecia, cancer cells appear to be greatly weakened in their defense against x-rays and become readily destroyed in the presence of porphyrin. Since only low dosages are required for their destruction, there would be less damage to non-cancer cells which do not take in the drug (absence of radiation burns to healthy tissue).

In these experiments, the porphyrin was injected by Dr. Figge prior to the radiation treatment. Some evidence was obtained to show that the tumors in porphyrin treated animals were more sensitive to radiation than the tumors containing less porphyrin. Our paper will show that porphyrin does indeed sensitize to x-radiation and it is able to alter this sensitizing influence on isolated cells such as Paramecium.

On the other hand, certain drugs are claimed to give preprotection against radiation damage. This feature is, of course, of great importance to the Navy since well characterized experiments showing the value of such reagents may afford great protection against radiation damage as in atomic and nuclear fission explosions.

Sodium pentobarbital and sodium nitrite, while they may exert a protective influence from radiation in multicellular organisms in the concentrations employed, did not protect paramecia from the lethal effects of radiation.

In this regard, it has been claimed by some that cysteine hydrochloride may give preprotection in certain multicellular organisms. Experiments were performed this summer to test this reagent against x-rayed paramecia. One per cent solutions were made to give dilutions of 1:5000 and 1:10,000 with sterile lettuce infusion when it was determined after many test concentrations that these dilutions were non-toxic to controls and believed to be closest to maximal effect on paramecia. With an original pH of 1.9, the solutions were buffered to pH 6.9 - 7.2 and considered satisfactory for paramecia. It is known that cysteine activates enzymes, combines with hydrogen peroxide, which is known to be formed in irradiated solutions, and rapidly oxidizes to the non-protective SS- cystine. As compared with the controls, the cysteine hydrochloride did not appear to give any appreciable protection to x-rayed paramecia when irradiated up to 500,000 roentgen in steps of 50.00 r.

Fig. 1. Machined Plexiglas cell (thickness, 2.5 cm.) showing deep well at each end for cracked ice and four Nylon syringes (2 cc capacity) each with Lucite cap. The Nylon syringes, which are used as irradiation chambers, contain 200 cells each and all syringes are X-rayed simultaneously. Two holes on each side of the Plexiglas cell permit rapid removal of syringes for cell-sampling in observing biological effects of x-radiation. (Approx. x1.)

(Plexiglas cell and Lucite caps made under direction of author by Mr. Michael Troisi, Instrument Maker, Temple University.)

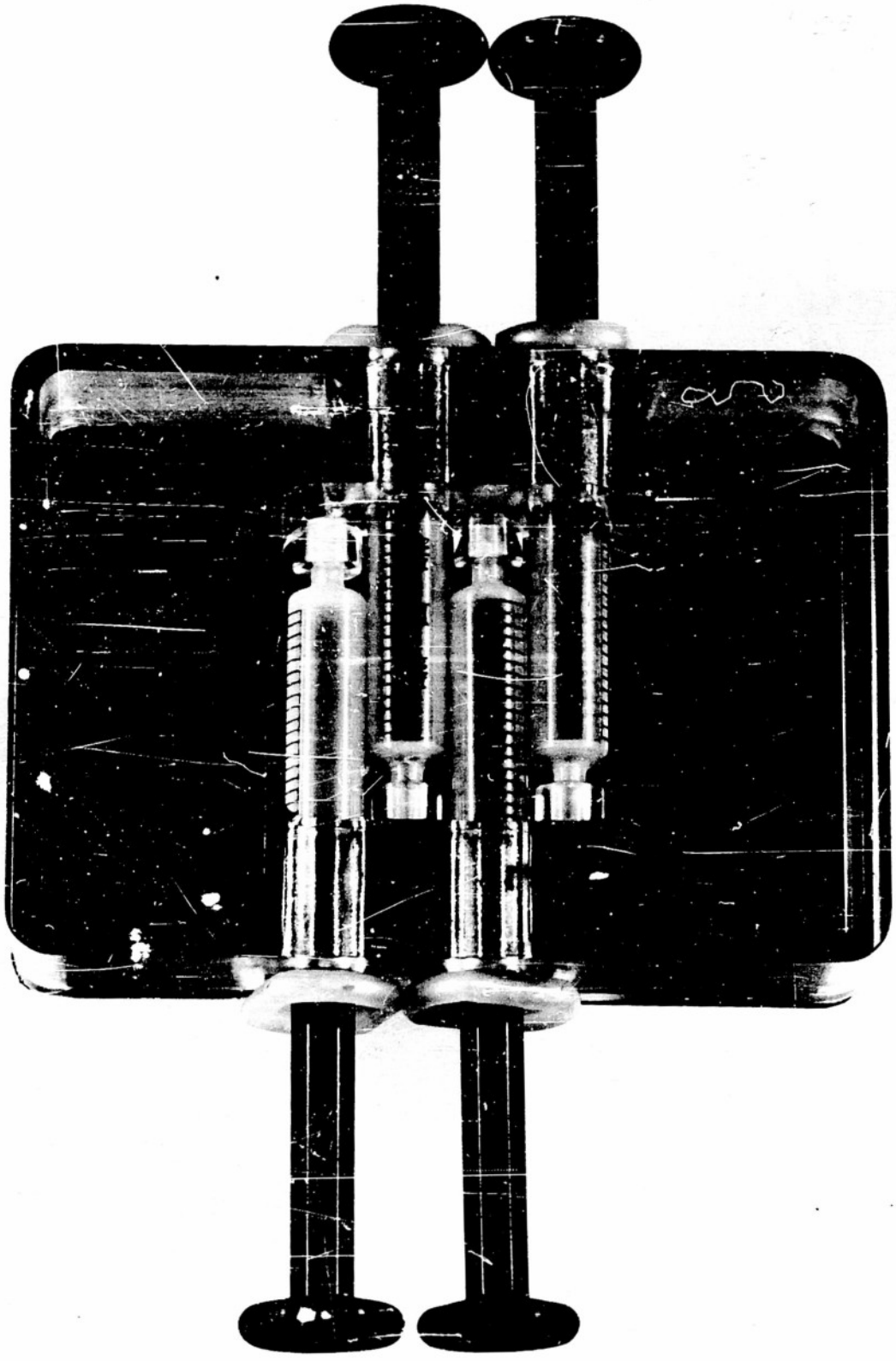

FIG. 1

Fig. 2. Effects of high dosage x-radiation on Paramecium caudatum. A. Unirradiated control specimen. B. Irradiated with 255,000 r resulting in slight change of body shape; animals generally recover from this dosage. C. Irradiated with 340,000 r (approximately the LD 50 dosage) in which locomotion and cyclosis are retarded. D. Irradiated with 425,000 r in which body shape becomes broadly ellipsoidal; greatly decreased locomotion; vacuolization. E. and F. Irradiated with 510,000 r resulting in cessation of locomotion and cyclosis, increased vacuolization, blistering of the pellicle, darkening (coagulation) of protoplasm followed by disintegration and death. (Photographs taken of irradiated specimens immediately after removal from x-ray generator.)

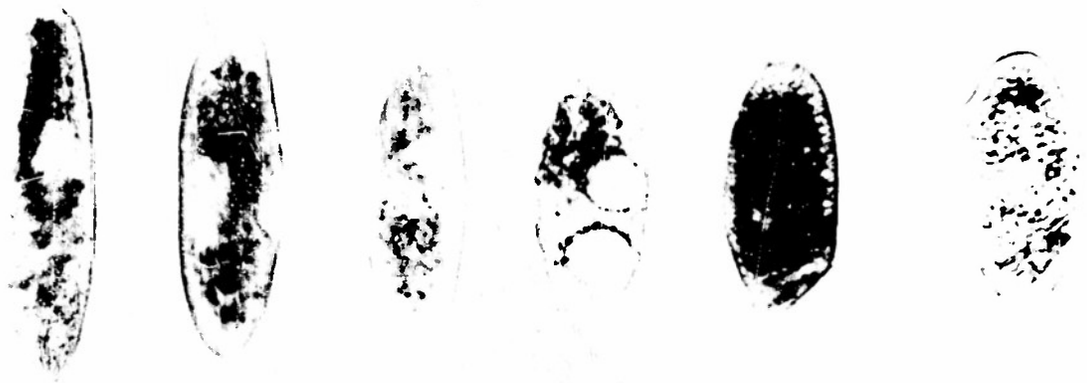

FIG. 2